Armand Maggenti

General Nematology

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With 135 Figures

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Front cover illustration. Left: Heth, a parasite of myriapods and diplopods. The genus was first described by Cobb in 1898. Right: A composite, three-dimensional drawing of a plant parasitic nematode.

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Preface

This text is an overall view of nematology because I believe the science should be treated as a unified discipline. The differences in the biological habits of nematodes do not justify the separation of plant nematologists and animal nematologists, since the separation is not a reflection of any differences inherent to nematodes. Therefore, the book is arranged with a format that in the beginning chapters illustrates the similarities and sequence of development of morphological characters among nematodes regardless of their biological habits. The later chapters illustrate the integration of the evolutionary development of the parasitic habit from related free-living forms.

Nematology is probably the last major discipline to establish its independence from the parent science of zoology. This natural evolvement of nematology has occurred because of the overwhelming accumulation of sophisticated information and research that reflects the unique relationships of nematodes to other forms of plant and animal life as well as their relationships in other facets of the environment. Nematodes are invertebrate animals that, like insects, are unusual in their great numbers and varieties, their small size (generally microscopic), their high degree of internal organization, and their virtually ubiquitous distribution. They occupy almost every ecological niche, often causing disease of humans, other animals, and plants. These activities often result in debility, death, or in the impairment and loss of food supply with consequent loss to producers and consumers.

Hopefully this book will intrigue teachers, students, nematologists, plant pathologists, parasitologists, and zoologists. Each will approach the book from their own level of needs; some will read it superficially, some will delve into its speculation, and all, I hope, will learn to appreciate the science itself.

In presenting my understanding of nematology, I hope to comply with the admonition of Thomas Huxley: "My business is to teach my aspirations to conform themselves to fact, not to try and make facts harmonize with my aspirations. . . . Sit down before fact as a little child, be prepared to give up every preconceived notion, follow humbly wherever and to whatever abysses nature leads, or you shall learn nothing." May the reader approach my effort with the same attitude.

I wish to acknowledge but not blame for this effort: D. J. Raski, who first introduced me to nematology; M. W. Allen for his teaching; and more immediately my wife Mary Ann to whom the book is dedicated, who put aside her own work to bring this book to fruition; W. H. Hart, Nahum Marban-Mendoza, and Ella Mae Noffsinger for their reviews and comments; Gaylen Paxman, nematologist, librarian, critic, and friend; my graduate students Fawzia Adbel-Rahman and Steve Martinez, who were interested and involved; all my students in Introductory Nematology, on whom the concepts presented here were first tried; R. Giblin; I. Cid del Prado; and especially my son Peter, who in critical moments eased the pressure by preparing pencil sketches for inking. My obligation is unbounded to B. G. Chitwood. He is a constant stimulus and his memory an abiding inspiration.

In 1967 B. G. Chitwood offered me the opportunity to revise the classic *An Introduction to Nematology* (B. G. and M. B. Chitwood). I felt then and I still believe that classic should stand. To modify it is to redo the Mona Lisa in crayons. I urge all students of nematology to read, read, and reread Chitwood. To have known the man was a pleasure, to have had him as a mentor exasperating, to have had him as a friend magnificent.

This is a short preface because the purpose of the book is to read it. Enjoy now these magnificent animals as I have.

Davis, California
July 1981

ARMAND MAGGENTI

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History of the Science

I. Introduction

> "The trails of the world be countless, and most
> of the trails be tried;
> You tread on the heels of the many, till you
> come where the ways divide;
>
> By the bones of your brothers ye know it, but
> oh, to follow you're fain.
> By your bones they will follow behind you, till
> the ways of the world are made plain."
>
> Robert Service

Too often history is considered an irrelevant study, but this is an injustice to past and present contributors of knowledge and experience. Paleontology is devoted to elucidating the history of animal and plant development on earth; so also is the history of a science an evolution of events leading to current concepts. Like a proper taxonomic classification, history allows us to understand not just the evolution of a phylum, but the internal individual phylogenies temporally and spacially. As Robert Service points out, the trails to truth are marked by the pioneers of the past, and those of the future by our own works.

Nematodes are the most numerous multicellular animals on earth. No other group of animals, save Arthropoda, have had such an impact on humans either directly or through agriculture. Nematodes are categorized as being free-living in a marine, freshwater, or soil environment, and as parasites of plants and animals. Often those working with plant parasites also study the free-living forms. Historically, the animal parasitologists were separated from nematologists as helminthologists, but this is now changing, thereby bringing the science of nematology into unity. Subjects such as taxonomy, morphology, and physiology require that all information be integrated and not fractionated. Cobb realized this and was the first to suggest that we call ourselves nematologists. With this proposal he acknowledged nematology as probably the last zoological science to deserve separate distinction.

The history of nematology, in this text, is not limited to this chapter. Readers will find many events and stories related in chapters throughout the book, wherever the telling is more relevant.

II. Ancient Times to the Eighteenth Century

The earliest reference to nematodes relates to animal parasites, specifically human parasites. Most often recorded were the large parasites visible to the naked eye. It is interesting, but not surprising, that written references were made to nematodes some 2000 years ago in records recovered from the great prevailing civilizations of the Mediterranean, Middle East, and Orient.

A. China

The oldest reference to parasitic nematodes is found in Huang Ti Nei Ching or *The Yellow Emperor's Classic of Internal Medicine* from China ca. 2700 B.C. This account is quite sophisticated in as much as it designates foods to be avoided as well as symptomatology and treatment. The symptoms of the giant intestinal worm (*Ascaris*) are surprisingly accurate. Hoeppli and Ch'iang in 1940 translated the following: "The symptoms of the disease are cardiac and intestinal pain, malaise, moving masses in the abdomen with intermittent pain, sense of heat in the abdomen with thirst and salivation."

Chinese medicine developed along empirical lines with little change until recently. Their philosophies greatly influenced their medical approach and prevented advancement until the twentieth century. However, the remarkable observations, as well as the herbal and acupuncture remedies that were developed, were at a more sophisticated level than in the Western world for thousands of years. Chang Chi (or Chang Chung-ching) noted ca. 217 A.D. that "During ordinary abdominal pains, the pulse becomes feeble and thready. If, on the contrary, it is full and bounding, it indicates the sure presence of Huei Ch'ung [*Ascaris*] in the abdomen."

Perhaps even more startling is the report of Ch'en Yen ca. 1174 A.D. in his work *The Three Causes and One Effect of Disease*: "Some people become parasitized by worms through eating fruits and vegetables or animal's viscera, which contain their progenies." This is a remarkable acknowledgment and represents a concept not accepted until the nineteenth century in the Western world. Bear in mind that the belief in spontaneous generation prevailed until Louis Pasteur's experiments in 1864. It is only fair, however, to point out that others such as Redi, von Leeuwenhoek, Spallanzani, Cagniard-Laton, and Schwann had much earlier proposed that the theory of spontaneous generation be discarded but were unable to satisfactorily convince the scientific world.

B. Mediterranean and Middle East Civilizations

The oldest record of nematodes among the ancient civilizations of the Mediterranean and Middle East occurs in the Ebers' Papyrus dated 1553–1550 B.C. This legacy of an Egyptain physician, discovered by Ebers in 1872, indicates that *Ascaris* (the giant intestinal worm) and *Dracunculus medinensis* (the guinea worm) were known at the time. However, it should be pointed out that it is impossible to know with certainty whether the intestinal worm referred to is an *Ascaris*, hookworm, tapeworm, or some other helminth. In addition to symptoms of "bowel worm" an anthelmintic (a drug used against helminths) made from the bark of the pomegranate tree (*Punica granatum*) was prescribed for explusion of the worm.

The next references to nematodes are found in the Bible, and some interpret the passages of Moses relating to Hebrew Laws of sanitation and hygiene as emanating from his early learnings from Egyptian physicians about parasites. Moses' probable knowledge of the guinea worm is found in Numbers 21:6–9. This reference to the fiery serpent and the likeness Moses made by winding the serpent on a staff are believed to have served as an example for the people to extract the worm from their tissues by winding it around a stick. This method of extraction is still practiced in many parts of North Africa and the Middle East.

Moses not only categorized animals as "clean" or "unclean" on the basis of visible parasites, but warned the people to beware of infected water. Moses could not have been aware of *Cyclops* as the intermediate host of *Dracunculus* or of the schistosome cercaria (flukes) in water; however, he certainly would have seen the cloudlike release of nema larvae from dracunculoid tumors when infected people stepped into water.

However one interprets biblical accounts concerning parasites, there can be little doubt of the reference in Plutarch attributed to Agatharchides of Cnidus (181–146 B.C.). In this account he clearly describes the guinea worm: "the people taken ill on the Red Sea suffered . . . worms, upon them, which gnawed away arms and legs, (tumorous ulcers) and when touched, retracted themselves up in the muscles and there gave rise to the most unsupportable pains. . . ." It is from this record that the generic name *Dracunculus* is derived: Plutarch used the Greek *dracontia micra* meaning "little snake." These observations also lend credence to the fiery serpent being *Dracunculus* because the Bible states that the Israelites passed through the region of the Red Sea on their way from Hor to Oboth. It has been estimated that this migration could have taken up to 12 months, which corresponds to the developmental period of 10–12 months for the guinea worm.

There certainly was no great impetus for the physicians of the day to pursue greater knowledge of parasites, including nematodes. No parallel development of symptomatology or therapeutics, such as occurred in the Orient, was proceeding in the Western world. This period is characterized by writings and observations without investigation. This stagnation persisted into the Christian Era and did not change until the nineteenth century.

Some notable events during these times are worthy of mention. Hippocrates, ca. 430 B.C., was aware of nemas as parasites and was likely the first to record knowledge of the pinworm *Enterobius vermicularis*. In his *Aphorisms* he mentions the presence of worms in the vagina of women (a common occurrence with pinworm) and of similar worms in horses. The latter represents the first veterinary observation.

Aristotle also knew of nemas as parasites, especially *Ascaris* and the pinworm. Unfortunately, he stated: "These intestinal worms do not in any case propagate their kind." For the world of science this engendered the theory of "spontaneous generation." No one before or since has held such a disastrous influence over science. I do not believe that Aristotle desired to stifle scientific development, but he did, and science was plunged into a dark age for almost 2000 years. During these dark ages little advance was made and most observations were isolated and of little import. Celsus (53 B.C.–7 A.D.) distinguished roundworms from flatworms; Columella, ca. 100 A.D., mentioned an *Ascarid* from a calf, probably *Neoascaris vitulorum*; Galen (130–200 A.D.) was the first to record nematodes of fish; Vegetius (ca. 400 A.D.) was the first to mention the horse ascarid *Parascaris equorum*. This period of history terminates with Albertus Magnus (1200–1280 A.D.) who provided the first record of nematodes from birds, namely, falcons. The science of nematology was advancing slowly with nearly 100 to 200 years between finds.

The sixteenth century marks a rewakening of science, but discoveries were still 50 to 100 years apart—encouraging, but only barely an improvement. As Chitwood noted: "the period from this time (16th century) until the latter part of the 18th century may be regarded as the medieval period of our subject." Caesalpinus (1519–1630) discovered the giant kidney worm *Dioctophyma renale* from a dog kidney. This parasite (1 m × 1.5 cm) remained the largest known nema until *Placentonema gigantisma* (8 m) was discovered by Gubanov in 1951 from the placenta of a sperm whale. Vinegia (1547) was the first to discover a filarid (Spiruria) in birds, again a falcon, as well as two intestinal nematodes.

III. History and Development in Nineteenth and Twentieth Century Europe

The major contribution to the advancement of nematology was the microscope. Tyson (1683) broke from the traditional recording period and was the first to study nemic anatomy and describe a nematode egg. Borellus (1656) discovered the first free-living nematode. From this period the science began to flourish both among zoologists, whose interest was concentrated on the free-living forms, and parasitologists who now could observe the lesser nemas. It was in this era that the first plant parasitic nema was discovered by Needham (1743) in wheat. This nema (*Anguina tritici*) continues to inflict economic losses in many regions of the world.

This early and exciting phase of discovering a wondrous variety of nemas shifted in the nineteenth century to anatomical studies by Bojanus (1817–1821) and Cloquet (1824), and life history and transmission studies by Owen (1835) and Leidy (1846). Owen discovered trichinosis and Leidy showed the role of rats and pigs in the transmission of the disease. These discoveries generated further research into nemic biology and in the waning years of the nineteenth century such startling discoveries as alteration of generations between free-living and parasitic phases were elucidated by Leuckhart (1865) and Metchnikoff (1865). These discoveries led, in turn, to the discovery that invertebrates often act as intermediate hosts for nemic parasites of higher vertebrates and humans. As a result, the mystery of the transmission of the fiery serpent (*Dracunculus*), which had eluded science for thousands of years was solved by Fedtschenko (1871) when he discovered that the small aquatic crustacean *Cyclops* was the intermediate host and that the disease was transmitted by drinking water contaminated with these animals.

The science of nematology finally took its rightful place among the other zoological sciences of Europe. In addition to biological studies, the art of taxonomy continued to flourish and was upgraded by scientists such as Dujardin (1845), Deising (1851), and Schneider (1866). Outstanding among these was Bastian who in 1865 described 100 new species of free-living nematodes. This work was followed by Bütschli and de Man.

Bütschli was not only a distinguished nematologist, but also a histologist whose development of paraffin embedding for thin tissue sections opened an entirely new avenue of research for all biological sciences.

Bütschli (1875) is also credited with the first observation of polar body formation during subdivision of the nucleus of the ovum. Nematodes then proved to be useful tools for the study of embryology and genetics. Van Beneden in 1883 discovered the mechanism of Mendelian heredity. Other contributors to the line of research were Boveri, zur Strassen, Martini, Müller, and Pai.

A great discovery for Nematology and all parasitology was made in 1878 by Manson who discovered that mosquitoes were the intermediate hosts and vectors of elephantiasis (*Wuchereria bancrofti*). This discovery was a significant catalyst to the discovery of mosquitoes as vectors for such diseases as malaria and yellow fever.

Plant nematology was not idle during this time either. Probably most significant to its growth and recognition was the introduction of sugar beets into Europe. It was not long until the industry was experiencing severe economic losses. The condition was not immediately attributed to nematodes, but to "beet tired soil." In 1859 Schacht discovered that the decline in beet production was always associated with a cyst-forming nematode now recognized as *Heterodera*. However, the nema was not accepted as the causal agent for some time and was first described and named by Schmidt in 1871. The official name became *Heterodera schachtii* after the original discoverer. During this period, the golden nematode of potato was also first seen, but not recognized as a separate species.

Investigations directed toward controlling these nemas dominated plant nematological research in Europe from 1870 to 1910. The control developed for sugar beet nematode is still used, i.e., crop rotation. The first trials to control nemas with soil fumigation were attempted by Kühn (1871) using carbon disulfide. Kühn also studied the feasibility of trap crops, which are plants that attract the nema but in which it cannot develop, or plants that are removed after invasion but before egg laying begins.

Root-knot nematode was first recognized in 1855 when it was discovered by Berkeley on cucumbers in an English glasshouse. This discovery soon led to the recognition of other plant parasitic nemas.

An important contribution, often overlooked, was made by Oerley in 1880, who in a compilation paper describing 202 nematodes in 27 genera, organized nemas into a classification and gathered related genera into families, most of which stand today.

In the late nineteenth and early twentieth century nematology experienced rapid growth and attracted some of the great biologists of Europe. One cannot read and study the works by Goldschmidt in the early 1900s without absolute amazement. His work on the nervous system remain magnificent monuments to his genius. Only now, with the electron microscope and computer analysis of sections, is similar work being produced. What is amazing is that very little is shown to differ from Goldschmidt's findings with the light microscope and paraffin sections.

In the 1930s two outstanding Russian scientists, Paramanov and Filipjev, were influential in establishing philosophies that brought nematology to maturity as a zoological science. Paramanov was a theoretician devoted to the evolutionary concept and the first to offer hypotheses concerning nemic relationships, evolution, and phylogenies. Many of his proposals still form the basic foundation of our understanding of nematode evolution. Filipjev was a taxonomist and offered a sound classification that was not widely accepted outside of Russia, but now forms the basis of current classifications of Nemata.

This is a brief history of the development of nematology in Europe and all contributors cannot be discussed. Among the many who should be remembered are Schuurmans Stekhoven, Jr., de Coninck, Steiner, Fuchs, and Goffart. In addition T. Goodey deserves special mention because his books still serve the science and he was instrumental to the development of nematology in Great Britain.

IV. History and Development in America

The first active study of free-living nemas in America was conducted by Joseph Leidy in 1851, but it was not until 1889 that the science really obtained some national recognition. The source of this impetus came from J. C. Neal and G. F. Atkinson who independently published on root knot in America. Neal's work covered the then active agricultural areas of the United States and this publication is well worth reading. In it Neal notes that, though not recognized by the scientific community, the presence of root knot in Florida extended back as far as the early Spanish explorers.

The most important person to the development of nematology in America was N. A. Cobb. His scientific contributions are notable, but even more important to the science was his personality. Cobb publicized nematology and obtained independent recognition for the science in the United States Department of Agriculture. His first paper on nematodes in America was published in 1913. A few years later he became associated with W. E. Chambers, whose illustrations distinguish Cobb's papers from all others. The quality of Chambers' art has never been equaled; however, they had a profound influence on the quality of illustrations by nematologists throughout the world. Because of Chambers the best illustrations among nematologists are produced by those workers interested in free-living and plant nematodes. Seldom do those who work with animal parasites achieve the quality so common in these other branches of the science. As a result, the classification and the species identification of animal parasites is chaotic.

Cobb surrounded himself with a nucleus of people who were the real founders and architects of the science in the United States, people such as A. L. Taylor, B. G. Chitwood, G. Thorne, J. R. Christie, and G. Steiner. Chitwood is likely the most outstanding nematologist of all time. Few even comprehended the broad scope of the science as he did. His book *An Introduction to Nematology, Section 1* is a classic; the information is as relevant today as when he first published the book in 1937. It combined personal research with the most complete compilation of nematode knowledge ever put together by an individual. A problem that evolved from this book was the overshadowing of Filipjev's classification. However, that seems to have been reversed in more recent years. Chitwood's contribution was to the whole science and not to any particular branch. The only comparable work is *Traité de Zoologie, Tome 4, Fasicule 2–3* edited by Pierre P. Grassé, published in 1965.

Through socratic teaching, these scientists, inspired by Cobb, trained the nematologists of the 1930s, 1940s, and 1950s. In the spring of 1948 M. W. Allen, trained by G. Thorne, taught the world's first formal university course in nematology at the University of California at Berkeley. Among the students in the first class were H. Jensen, of Oregon State University, and W. H. Hart. Hart in 1951 was the first nematologist appointed to a state position in the California Department of Food and Agriculture. In 1959 Hart moved to the University of California Cooperative Extension Service as the first specialist in nematology. Allen was also instrumental in 1954 to the forming of the first Department of Nematology at a university. The first chairman of the statewide department was D. J. Raski at the University of California at Davis with branch departments at Berkeley and Riverside. Since that time classes and departments devoted to the science have formed throughout North America.

A period of great expansion occurred after World War II. The impetus came from the discovery by W. Carter in the early 1940s of a safe, economical, and highly effective soil fumigant. This allowed nematologists to demonstrate with practical field control the great economic losses that were occurring in agricultural crops. The fumigant was a dichloropropene–dichloropropane mixture, which in a refined state is still extensively used throughout the world.

Chapter 2
Nematodes and Their Allies

The relationship of nematodes to other organisms remains unclear even after 100 years of zoological arguments. Nematodes have been assigned to no less than four phyla. Perhaps the most generally accepted has been that of Aschelminthes, Grobben 1909. This group, adhered to by Hyman, includes 6 classes: Rotifera, Gastrotricha, Kinorhyncha (Echinodera), Priapulida, Nematoda, and Nematomorpha. The Priapulida, because their musculature is longitudinal and circular and because of variations in their body cavity, have been excluded from the phylum Aschelminthes. In other schemes, nematodes are placed in the phylum Nemathelminthes, which generally includes just the Nematoda and Nematomorpha, thus leaving Aschelminthes to hold Rotifera, Gastrotricha, and Kinorhyncha. In this text I will hold to the concept that nematodes belong in a phylum of their own, Nemata, as first proposed by Cobb in 1919, and reinstated by Chitwood in 1958; and that each of the so-called related groups, that is, rotifers, gastrotrichs, kinorhynchs, and nematomorphs, are to be placed in their own separate phyla.

The primary reason for disagreement arises from the concept of the pseudocoelom (body cavity). The pseuodcoelom is a nonmorphological zoological term. The fact remains that probably no other structure has been submerged in more vagueness, pseudodefinitions, and misinterpretations than the coelom, whether it is pseudo (partially lined by mesoderm), or true (completely lined by mesoderm). It is distressing that in the past 100 years we have learned nothing more about the embryological development of the so-called body cavity of pseudocoelomates. Some believe that it is a remnant of the original blastocoel, others define it as a gymnocoel, and still others put it in the classification of a mesenchymocoel or schizocoel.

The **blastocoel** is the primary cavity formed during the embryological development of animals. It is believed by some that in the pseudocoel groups the blastocoel persists into the adult animal. A **mesenchymocoel** is a body cavity within a mesenchymal mass of tissue. A **schizocoel** is a body cavity formed by spaces within a compact tissue of the embryo. A **gymnocoel** is a body cavity that has no special lining cells other than tissues bordering cavities such as epidermis or gastrodermis. There is little evidence to indicate that the pseudocoelom evolved in exactly the same manner embryologically in these diverse groups of animals with some superficial resemblance. The known embryology of the various groups indicates that each group has a rather distinct type of body cavity. Nematomorphs are filled with a mesenchyme-like tissue. Nematodes have a well-developed body cavity filled with fluid and with some evidence of mesodermal lining, if one considers the muscle sheath as mesoderm, and the epidermal layer around the gonads and the basal lamella of the intestine as being of mesodermal origin. In gastrotrichs each class differs in the type and manner of body cavity formation as well as in the number of cavities included within the body. It may be that all these embryological phenomena occur within and among these group. If this were so, this would further support the independence of these groups.

Most of the characteristics used to define the groups such as Aschelminthes are rather superficial, such as the **protonephridial** excretory system (an excretory system composed of tubules ending in flame cells), which is present in all the groups save one class of gastrotrichs and the entire Nemata. There is no evidence that the ventral excretory cell seen in Nemata has any relationship to a protonephridial excretory system.

The bodies of members of all these groups are covered by a noncellular elastic cuticle and this is given as a point of relationship, but the formation of the epidermis underlying this cuticle differs among all the groups. In most nematodes it is composed of discrete cells whose cell bodies lie in chords laterally, ventrally, and a portion of the body dorsally. In other pseudocoelomates the epidermis is syncytial (multinucleate), and occasionally discrete uninucleate cells are present, but seldom do they lie in chords in the same fashion seen in nematodes. The musculature of the various "Aschelminthes" also differs. There is no complete muscle sheath in rotifers, gastrotrichs, or kinorinchs. In these animals muscles are limited to scattered bands.

Most of the characteristics considered to show a relationship are not a function of evolutionary sequence but rather of evolutionary demands because small animals have problems in compensating surface area to volume. Small animals also are limited in the number of cells they can contain. Therefore, variety and modification based on these two restrictive elements allow for few or bizarre variations. The larger the animal the greater become the demands on surface-dependent functions. The coelom is necessary not only for complex locomotion, but also for an increase in size and cell numbers. Animals compensate for an increase in size in three general ways: They can differentially increase the surface body area by